

# Genetic Relationships (RAPD-PCR) Between Geographically Separated Populations of the “Cosmopolitan” Interstitial Polychaete *Hesionides gohari* (Hesionidae) and the Evolutionary Origin of the Freshwater Species *Hesionides riegerorum*

HARTMUT SCHMIDT AND WILFRIED WESTHEIDE\*

*Spezielle Zoologie, Biology/Chemistry, University of Osnabrück, 49069 Osnabrück, Germany*

**Abstract.** In an analysis of the population genetics of the tiny meiofaunal polychaete *Hesionides gohari*, the RAPD-PCR method was applied to 49 specimens from 7 collecting sites far apart on three continents: French Atlantic coast, Mediterranean (Majorca, Giglio, Crete), Red Sea, Indian Ocean (Phuket), and U.S. Atlantic coast (Florida). In the band patterns produced with 14 arbitrary decamer primers, 496 genetic characters were detected. Genetic distances between the *H. gohari* populations vary between 0.55 and 0.70. The data were evaluated by three cluster programs: in the almost congruent phenograms, three clades were found with high bootstrap values: (1) European Atlantic–Mediterranean–Red Sea, (2) Indian Ocean, (3) Western Atlantic. In all cluster analyses, *Hesionides riegerorum* from a U.S. east coast river system is shown as genetically nearest to the Florida specimens of *H. gohari*, making it most probable that this freshwater species of the genus originated from a Western Atlantic *H. gohari* population. The genetic distances detected between the *H. gohari* specimens from the three continents are almost identical to those found between morphologically similar interstitial polychaete species pairs. Thus, the degree of genetic consistency is considered not to be high enough to corroborate the notion of a cosmopolitan distribution pattern, but rather suggests that the three clades represent different species.

## Introduction

The many species of *Hesionides* Friedrich, 1937, are highly characteristic meiofaunal polychaetes with a great variety of morphological, reproductive, and behavioral adaptations to the habitat of interstitial crevices in marine surf-beaten sand beaches (Westheide, 1967, 1971, 1984). Several of the *Hesionides* species are known to be characterized by another peculiarity of the marine interstitial fauna—a broad geographic distribution, comprising intertidal localities throughout the world (Westheide, 1971; Sterrer, 1973; Giere, 1993). Within the genus, *H. arenaria* Friedrich, 1937, and *H. gohari* Hartmann-Schröder, 1960, appear to have the most cosmopolitan distribution (Westheide, 1977); they occur on all continents except Antarctica. *H. gohari* was discovered by Adolf Remane in littoral sands near Ghardaqa (today: Hurghada) in the Red Sea (Remane and Schulz, 1964); it was described by Hartmann-Schröder (1960). Westheide (1970, 1972a, 1972b) investigated the morphology, reproductive biology, and local distribution pattern of populations occurring in beaches of northern Tunisia (Mediterranean Sea).

This tiny species (max. length 1.5 mm) is typical of warm seas and has a range extending as far north as Arcachon, on the Atlantic coast of France (Westheide, 1972/73). New localities for the species are still being recorded worldwide (Hartmann-Schröder, 1991; Westheide, 1992). Its most characteristic diagnostic features are considered to be its length, the proportions of the head appendages, the position of penis papillae, the dentation of notopodial chaetae, and especially the shape of the anal appendages. Among the other *Hesionides* species of comparable size, one inhabits

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\* To whom correspondence should be addressed. E-mail: westheide@mail.biologie.uni-osnabrueck.de

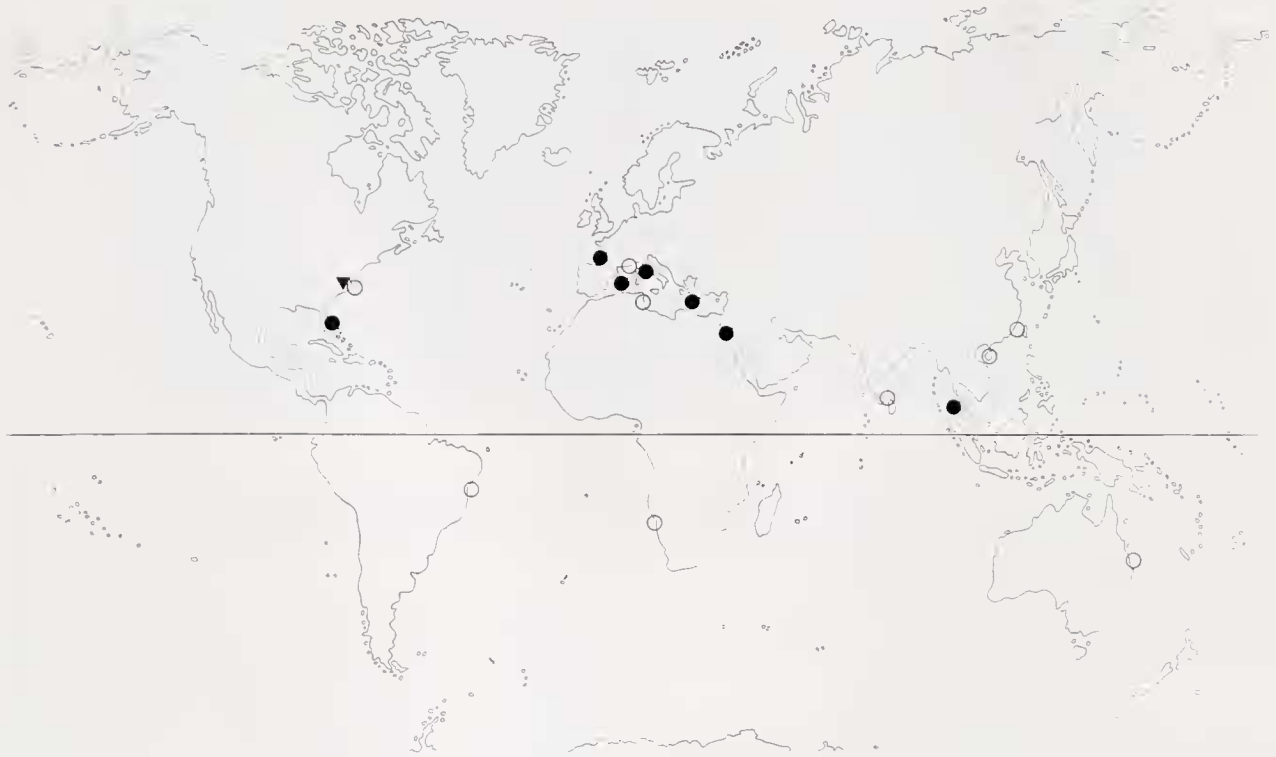


Figure 1. Records (open circles) and sampling sites (solid circles) for *Hesionides gohari* and sampling site for *H. riegerorum* (triangle).

fresh water. *H. riegerorum* Westheide, 1979; it can be distinguished from *H. gohari* by various morphological features.

The demonstrated almost cosmopolitan distribution of these *Hesionides* species, together with that of many other species of various meiofaunal taxa, gave rise to a still-controversial hypothesis of Sterrer (1973) (see also Rao, 1972) based on the notion that speciation of these forms is extraordinarily slow. It is postulated that they were already present on an old supercontinent and were distributed over their present vast range by the drifting apart of the continental plates. This explanation could apply only if the genetic changes in the separated populations were relatively small over long periods of time, and mostly had no effect on the phenotype. Such minimal genetic variability can be explained by assuming a complete constancy of the ecological factors in the habitat of these animals, the sand beaches. Sterrer's hypothesis appeared necessary because long-range, transoceanic dispersal of these meiofaunal organisms seemed inconceivable: they are not capable of active swimming and, with few exceptions, have no larval dispersal stages.

However, it gradually became evident that, even without dispersal stages, many of these species do succeed in colonizing geologically young islands far from any coast (Westheide, 1991); furthermore, dispersal of meiofaunal individ-

uals in the seawater column along shores has been observed and demonstrated experimentally (Hagerman and Rieger, 1981; Palmer, 1988; Armonies, 1989). These observations led to the proposal that the cosmopolitan distribution pattern is ascribable not to geologically ancient processes but rather to occasional contemporary events (Sterrer, 1973; Gerlach, 1977) involving long-range dispersal by birds, on drifting material, or in ballast sand or tanks in ships.

Lately, arguments for one or the other view have been supported by genetic investigations. Todaro *et al.* (1996) expressed the conviction, based on their restriction-fragment length polymorphism analyses, that the proposed cosmopolitan species *Xenotrichula intermedia* (Gastrotricha), apparently present both in the Mediterranean Sea and along the coast of North America (Ruppert, 1977), consists of more than one taxon. Similarly, with RAPD-PCR (random applied polymorphic DNA-polymerase chain reaction) Soosten *et al.* (1998) found genetic differences between specimens of the polychaete *Petitia amphophthalma* from Europe and North America, although these differences are considerably smaller than those between certain morphologically distinct species of other taxa. In the present study of *Hesionides gohari* collected from seven intertidal localities on three continents (Europe, North America, Southeast Asia; Fig. 1), the RAPD-PCR method was likewise chosen, to evaluate the results obtained for *P. amphophthalma* with

Table I

Collection information for *H. gohari* and *H. riegerorum*

Species	Locality		Abbreviation	Number of specimens	
<i>H. gohari</i>	Atlantic Ocean	France	Arcachon (intertidal)	A	11
<i>H. gohari</i>	Mediterranean Sea	Spain, Majorca	Palma (subtidal)	M	8
<i>H. gohari</i>	Mediterranean Sea	Italy, Giglio	Campese (subtidal)	G	8
<i>H. gohari</i>	Mediterranean Sea	Greece, Crete	Heraklion (intertidal)	C	8
<i>H. gohari</i>	Red Sea	Egypt	Hurghada (intertidal)	H	4
<i>H. gohari</i>	Indian Ocean	Thailand	Phuket (intertidal)	T	4
<i>H. gohari</i>	Atlantic Ocean	Florida	Ft. Pierce (intertidal)	F	6
<i>H. riegerorum</i>	Chowan River	North Carolina	Edenhouse (riverbank)	NC	5

those of other cosmopolitan species studied in our laboratory (Schmidt and Westheide, 1998; Schmidt unpubl. results). For general advantages of the RAPD-PCR method in molecular systematic and ecological studies, see Hadrys *et al.* (1992), Schierwater (1995), and Schirmacher *et al.* (1998).

### Materials and Methods

A total of 49 individuals of *Hesionides gohari* Hartmann-Schröder, 1960, from seven localities and 5 specimens of *H. riegerorum* Westheide, 1979, from North Carolina were examined genetically (Table I). The meiofaunal animals were extracted from small samples of sand by the magnesium chloride method (Westheide, 1990). After sorting and washing in doubly distilled water, the specimens were either frozen in LTE-buffer at  $-60^{\circ}\text{C}$  or dried at temperatures below  $90^{\circ}\text{C}$ . Both methods of preservation guaranteed reproducible results even when samples were stored for months prior to actual DNA isolation.

Total DNA was isolated by a modification of the method of Kocher *et al.* (1989). The DNA was extracted from frozen tissues ( $-70^{\circ}\text{C}$ ) in 1.5-ml microtubes by digestion in 100  $\mu\text{l}$  100 mM Tris HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, 0.1% SDS, 50 mM dithiothreitol and proteinase K (0.5  $\mu\text{g}/\text{ml}$ ) incubated for 2 h at  $50^{\circ}\text{C}$  in a heating block. SDS was precipitated by 40  $\mu\text{l}$  3 M potassium acetate cooled down to  $0^{\circ}\text{C}$  for 10 min. The DNA was purified by extracting once with 150  $\mu\text{l}$  phenol/chloroform/isoamyl alcohol (25:24:1, saturated with 10 mM Tris, pH 8.0, and 1 mM EDTA) and once with 150  $\mu\text{l}$  chloroform/isoamyl alcohol (24:1). After precipitation with 150  $\mu\text{l}$  100% isopropanol, the DNA was washed with 100  $\mu\text{l}$  70% ethanol (both refrigerated at  $0^{\circ}\text{C}$ ). The phases were separated by centrifugation at 14,000 rpm for 10 min at  $4^{\circ}\text{C}$ . The dried DNA was dissolved in 1 mM Tris HCl, pH 8.0, plus 0.1 mM EDTA and stored at  $4^{\circ}\text{C}$ .

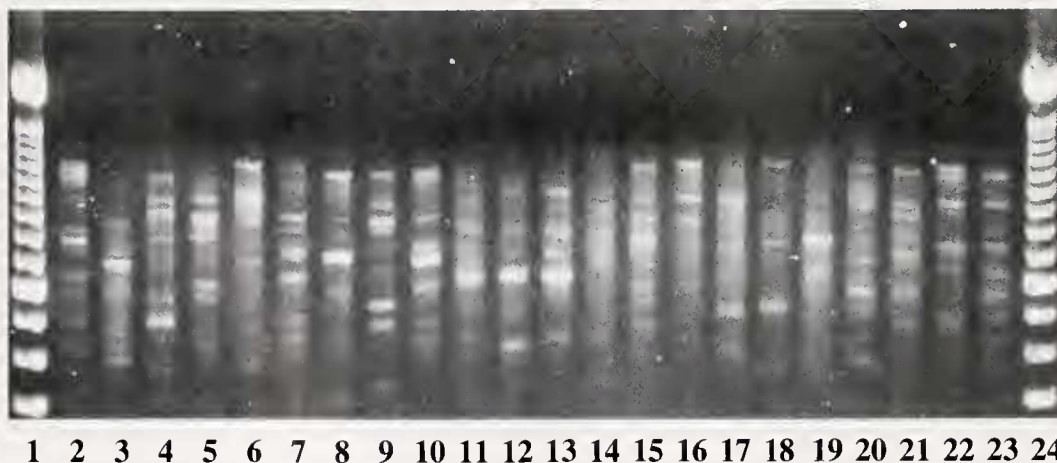
Fourteen arbitrary 10-mer primers from Operon Technologies (Alameda, California) listed in Table II were used for PCR amplification following the recipes reported by Wil-

liams *et al.* (1990) with minor modifications. PCR was done in a total reaction volume of 25  $\mu\text{l}$  with the following compounds: 10 mM Tris HCl, pH 8.3; 50 mM KCl; 2 mM  $\text{MgCl}_2$ ; 1% Triton X-100; 100  $\mu\text{M}$  each of dATP, dCTP, dGTP, and dTTP (Boehringer); 5 pM decamer primer; 1–5 ng DNA; and 0.4 U Tbr Polymerase (Biometra Prime Zyme™ Polymerase). Controls were run with no template DNA. Amplification was done simultaneously by two thermocyclers: Perkin Elmer Cetus DNA thermal cyclers 480 and Biometra personal cyclers 48. All synthesized fragments patterns were comparable. Each PCR cycle consisted of denaturation for 1 min at  $94^{\circ}\text{C}$ , hybridization for 1 min at  $36^{\circ}\text{C}$ , and extension for 2 min at  $72^{\circ}\text{C}$ . This cycle was repeated 45 times followed by a paused file at  $4^{\circ}\text{C}$ . Fastest available transitions between temperatures were employed in each case. Reaction products were separated by electrophoresis in 1.5% agarose gels buffered in  $1\times$  TBE (0.045 M Tris-borate, 1 mM EDTA) at 3 V/cm for 2.5 h, stained with ethidium bromide, and documented under UV light. As size markers, 100-base-pair DNA ladders were used (from Life

Table II

Decamer primers and their sequences used in this study (all primers from Operon Technologies, Inc., Alameda, CA)

Primer	Primer sequence 5' to 3'	Molecular weight
OPB-01	GTTTCGCTCC	2961
OPB-02	TGATCCCTGG	3010
OPB-03	CATCCCCCTG	2915
OPB-04	GGACTGGAGT	3099
OPB-05	TGCGCCCTTC	2946
OPB-06	TGCTCTGCCC	2946
OPB-07	GGTGACGCAG	3048
OPB-08	GTCCACACGG	3004
OPB-10	CTGCTGGGAC	3035
OPB-11	GTAGACCCGT	3019
OPB-12	CCTTGACGCA	2779
OPB-15	GGAGGGTGTT	3130
OPB-17	AGGGAACGAG	3117
OPB-18	CCACAGCAGT	2988



**Figure 2.** RAPD fingerprinting of *Hesionides gohari* specimens from Phuket (2, 3), Florida (8–11), Crete (12–15), Giglio (16–18), Arcachon (19–23), and *H. riegerorum* specimens from North Carolina (4–7); 1 and 24: 100 bp marker. Primer OPB 6.

Technologies, Inc., Berlin, Germany, or Pharmacia Biotech, Uppsala, Sweden). The reproducibility of the data was checked at regular intervals on different levels throughout all three parallel experiment lines. First, at the beginning of each series the optimal DNA concentration for PCR was determined for the individual animal, by testing the PCR with three DNA concentrations per animal (*ca.* 1 ng, 3 ng, and 10 ng per 25  $\mu$ l reaction volume). In pilot experiments with larger annelids, it became clear that the results were reproducible with up to 25 ng/25  $\mu$ l reaction volume. With DNA concentrations as high as *ca.* 50 ng/25  $\mu$ l reaction volume, reproducibility was distinctly worse. A further increase in DNA concentration (to over 100 ng/25  $\mu$ l reaction volume) brought the PCR to a complete halt, so that no specific DNA fragments could be detected. Reproducibility was tested further throughout each test series by running the same experiment twice in parallel reactions. In addition, for each series one reaction was carried out with a "blind" sample lacking DNA, to check the possibility of contamination with foreign DNA. We also looked for differences in the amplification patterns that might derive from the use of different thermocyclers (Büscher *et al.*, 1993; He *et al.*, 1994) or preservation methods (drying or deep-freezing), but found none.

The detected amplification product patterns were examined visually for monomorphic and polymorphic markers (Hadrys *et al.*, 1992). The degrees of polymorphism are given in percentages. The banding patterns were then translated into a 0/1-matrix (0 for absence, 1 for presence of a specific DNA marker) and fed into the cluster analysis program TREECON 1.2 (Peer and Wachter, 1994), which also transformed the data into distance values in percentages (Nei and Li, 1979). Cluster analyses were carried out by UPGMA (unweighted pair-group method using arithmetic averages; Sneath and Sokal, 1973), single linkage (Sneath

and Sokal, 1973), and neighbor joining (Saitou and Nei, 1987) including bootstrap proportions (Felsenstein, 1985).

## Results

For the seven sampled populations of *H. gohari* and one population of *H. riegerorum*, with 14 different primers a total of 496 different DNA fragments were detected, ranging in length from 150 bp to 1900 bp (*e.g.*, Fig. 2).

The calculated degree of polymorphism is similarly high in all the populations tested: for Arcachon, 96%; Giglio, 94%; Majorca, 95%; Crete, 94%; Hurgada, 89%; Phuket, 89%; Florida, 97%; and for *H. riegerorum* of North Carolina, 86%, the lowest value. For none of the European populations, nor for the population at the Red Sea, could diagnostic DNA fragments be detected. However, if the European and Egyptian animals are considered as a single group, its members are found to exhibit 33 common polymorphic characters that are absent from the individuals living in Thailand and Florida. With few exceptions, characters present in the animals from Thailand are the same as those found in the European and Egyptian individuals. Three diagnostic and five polymorphic DNA fragments were observed in the Phuket population only. Only one characteristic polymorphic character was detectable in the animals from Florida, and none of the primers revealed diagnostic DNA fragments. A surprising result was obtained by comparing the population of *H. riegerorum* with those of *H. gohari*. Although this freshwater species can be distinguished from the *H. gohari* populations by 12 diagnostic DNA fragments and 9 polymorphic characters, all other DNA bands are common to the two groups. There is an especially close match with the animals of the Florida population: of the 191 shared characters, 39 polymorphic DNA fragments are present only in these individuals. The

Table III

Comparison of genetic distances generated from RAPD data (after Nei and Li, 1979) within and between the seven *Hesionides gohari* populations and the sole population of *Hesionides riegerorum*

Locality	Arcachon	Giglio	Majorca	Crete	Hurghada	Florida	Phuket	N. Carolina <i>H. riegerorum</i>
Arcachon	$\bar{x} = 0.53$ 0.43–0.6							
Giglio	$\bar{x} = 0.57$ 0.48–0.67	$\bar{x} = 0.50$ 0.38–0.61						
Majorca	$\bar{x} = 0.58$ 0.51–0.66	$\bar{x} = 0.55$ 0.43–0.67	$\bar{x} = 0.53$ 0.45–0.61					
Crete	$\bar{x} = 0.56$ 0.48–0.63	$\bar{x} = 0.55$ 0.46–0.65	$\bar{x} = 0.55$ 0.47–0.61	$\bar{x} = 0.50$ 0.37–0.58				
Hurghada	$\bar{x} = 0.60$ 0.5–0.68	$\bar{x} = 0.59$ 0.53–0.64	$\bar{x} = 0.61$ 0.54–0.69	$\bar{x} = 0.6$ 0.54–0.68	$\bar{x} = 0.51$ 0.46–0.61			
Florida	$\bar{x} = 0.70$ 0.63–0.78	$\bar{x} = 0.70$ 0.6–0.77	$\bar{x} = 0.68$ 0.59–0.75	$\bar{x} = 0.68$ 0.62–0.75	$\bar{x} = 0.70$ 0.65–0.76	$\bar{x} = 0.58$ 0.51–0.56		
Phuket	$\bar{x} = 0.68$ 0.62–0.76	$\bar{x} = 0.65$ 0.56–0.75	$\bar{x} = 0.68$ 0.6–0.73	$\bar{x} = 0.64$ 0.57–0.74	$\bar{x} = 0.69$ 0.62–0.76	$\bar{x} = 0.70$ 0.64–0.77	$\bar{x} = 0.50$ 0.47–0.53	
N. Carolina <i>H. riegerorum</i>	$\bar{x} = 0.78$ 0.73–0.86	$\bar{x} = 0.79$ 0.70–0.87	$\bar{x} = 0.78$ 0.69–0.78	$\bar{x} = 0.77$ 0.71–0.83	$\bar{x} = 0.77$ 0.72–0.83	$\bar{x} = 0.68$ 0.56–0.77	$\bar{x} = 0.75$ 0.70–0.81	$\bar{x} = 0.40$ 0.28–0.47

Values are means ( $\bar{x}$ ) followed by min/max ranges.

average genetic distances and the range of variation within and between the seven *H. gohari* populations are shown in Table III, as is their genetic relation to the population of *H. riegerorum* in North Carolina.

The genetic distance values within the European, Egyptian, and Thai populations are roughly the same, but a somewhat higher value was obtained for the animals from Florida. The genetic distances among the individuals of *H. riegerorum* are distinctly less than the values for all the *H. gohari* populations. The European populations, including the animals from Hurghada, bear the closest genetic resemblance to one another, and each of them is about the same distance away from the population of Thailand and from that of Florida. Comparison between *H. riegerorum* and the *H. gohari* populations gave an unexpected result (Table III): whereas *H. riegerorum* is, unsurprisingly, relatively distant genetically from the populations of Europe, Egypt, and Thailand, its genetic distance from the population of Florida is less.

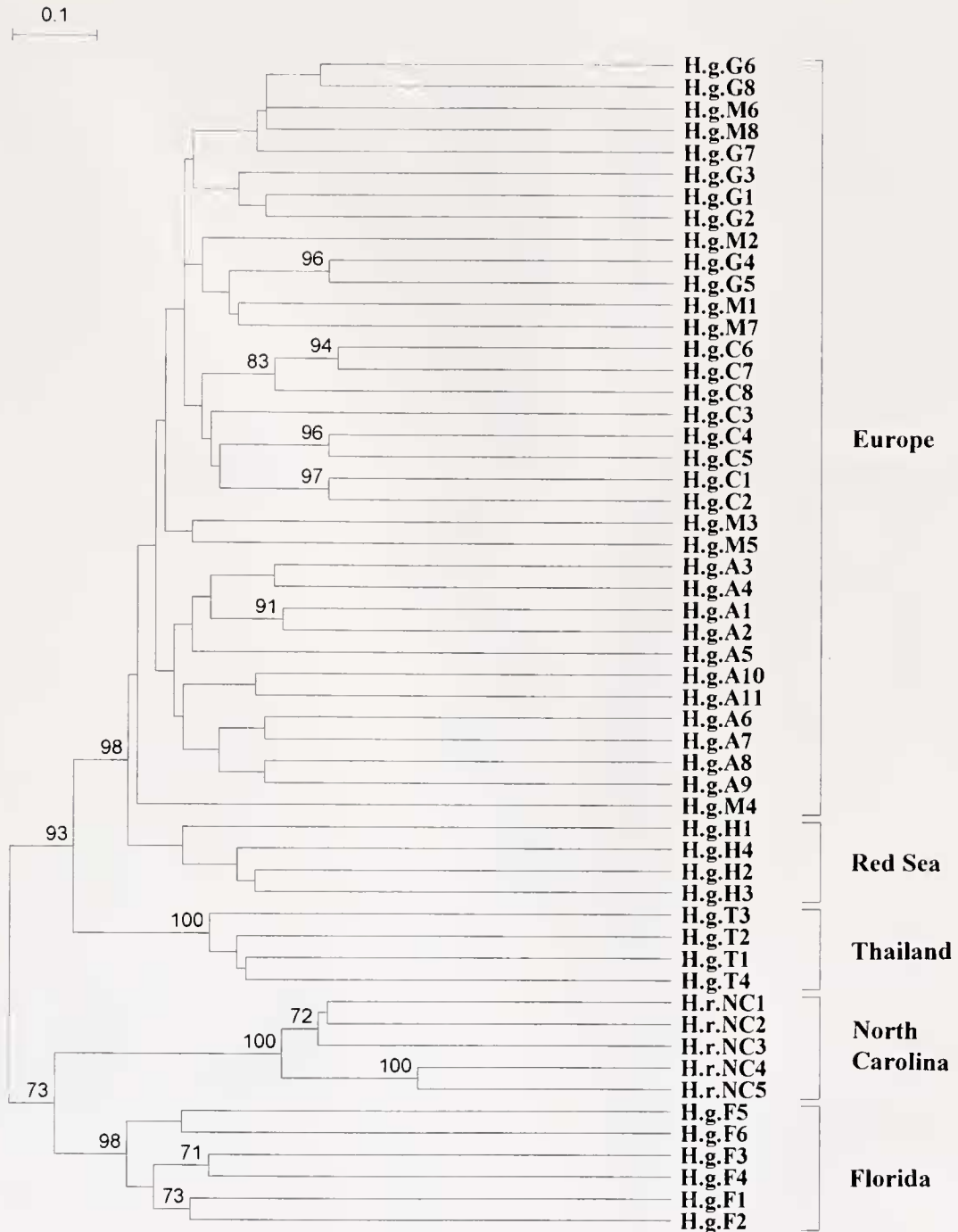
These findings become particularly clear when the associated phenograms are constructed (Figs. 3–5). With all three analytical procedures, the individuals of the following geographic regions form groupings with high bootstrap values: (1) European Atlantic coast (Arcachon)–Mediterranean (Majorca, Giglio and Crete)–Red Sea; (2) Indian Ocean (Phuket); (3) Western Atlantic coast (Florida); (4) *H. riegerorum* (Chowan River, North Carolina). The European *H. gohari*, including the individuals from the Red Sea, form a common cluster in the phenograms, the common root of which has a bootstrap value ranging from 76 (single linkage) to a maximum of 98 (UPGMA).

Only with UPGMA (see Fig. 3) can the animals from the Red Sea be distinguished as a cluster (bootstrap value <70); with neighbor joining (Fig. 4) and single linkage (Fig. 5) they fall within the European cluster but still form a bootstrap-supported cluster of Red Sea animals. The individuals from the French Atlantic coast form a common cluster (bootstrap value <70) only with UPGMA, and this cluster lies between the Mediterranean populations. In all the trees, the animals from Thailand are separate from the European–Egyptian group.

All cluster analyses distinguish *H. riegerorum* from the *H. gohari* of Florida, but together, the Florida *H. gohari* and the *H. riegerorum* always form a cluster that is separate from the individuals of the *H. gohari* populations. That is, according to the present results, animals living in regions close together geographically are genetically more similar, although they belong to two morphologically distinct species, than individuals that are considered to be of the same species but originate in regions far apart from one another.

## Discussion

The European populations of *H. gohari* treated in the present study (from Arcachon into the Mediterranean region) are relatively similar genetically, in some cases forming intermingled clusters, but clustering differently in the different analyses. It is therefore extremely likely that these individuals belong to a single species. The animals from the Red Sea form a distinct cluster in only one of these analyses; treated with either of the two other procedures, the four individuals in this sample are positioned among the Euro-

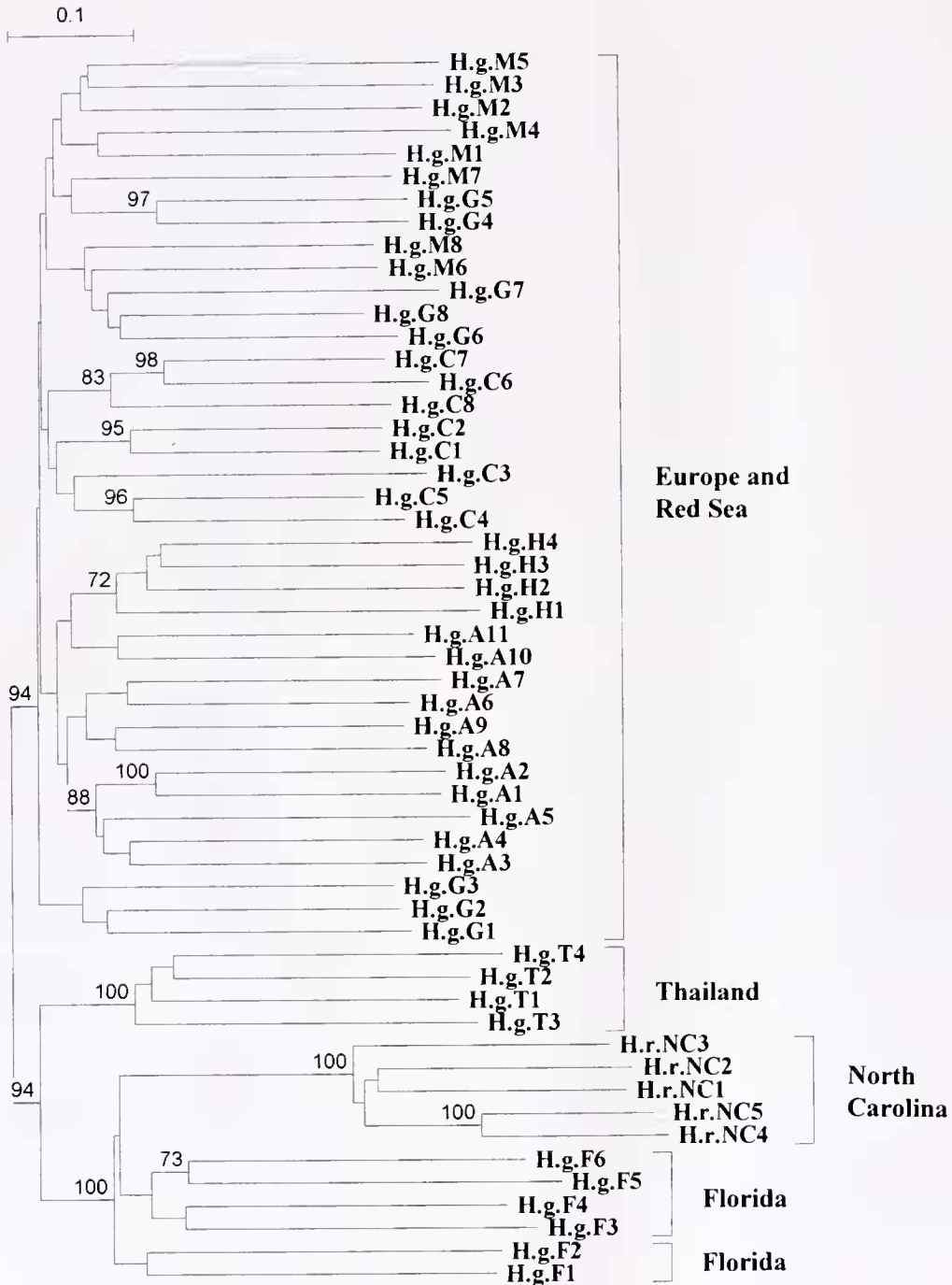


**Figure 3.** UPGMA phenogram of *Hesionides riegerorum* specimens (H. r.) from North Carolina (NC) and *H. gohari* specimens (H. g.) from the local populations in Arcachon (A), Giglio, Mediterranean (G), Majorca, Mediterranean (M), Crete, Mediterranean (C), Hurghada, Red Sea (H), Florida (F), Phuket, Thailand (T). Only bootstrap values above 70 are indicated.

pean individuals. Their genetic distances are generally not much different from those within the European group, so that they too are very probably members of the same species. The fact that the Red Sea site where they were collected, Hurghada, is the type locality of *Hesionides gohari*

Hartmann-Schröder, 1960, presumably makes it highly likely that all animals of this cluster are of this species.

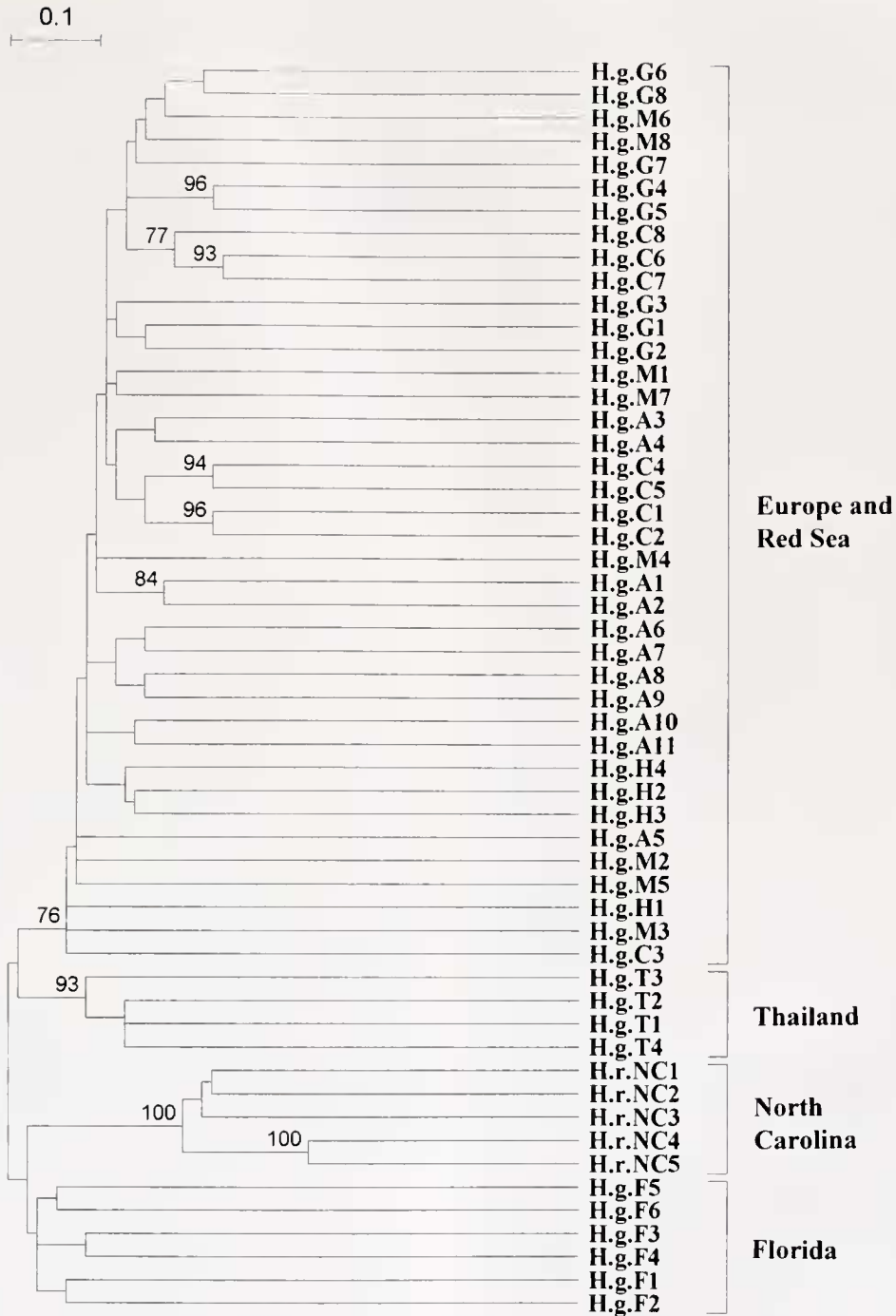
The relatively slight genetic distance between Red Sea animals and Mediterranean animals indicates either that gene exchange occurred between previously isolated popu-



**Figure 4.** Neighbor-joining phenogram of *Hesionides riegerorum* specimens (H. r.) from North Carolina (NC) and *H. gohari* specimens (H. g.) from the local populations in Arcachon (A), Giglio, Mediterranean (G), Majorca, Mediterranean (M), Crete, Mediterranean (C), Hurghada, Red Sea (H), Florida (F), Phuket, Thailand (T). Only bootstrap values above 70 are indicated.

lations in these two regions after the two seas were joined by the construction of the Suez Canal, or that one population was isolated from the other by migrating through the Canal after it was opened. The latter possibility is likely, but it is

hard to decide whether the migration was from the Red Sea into the Mediterranean and on to the Atlantic (Lessepsian migration; Por, 1978) or in the other direction. No finds from areas further east, between Hurghada and Thailand,



**Figure 5.** Single-linkage phenogram of *Hesionides riegerorum* specimens (H. r.) from North Carolina (NC) and *H. gohari* specimens (H. g.) from the local populations in Arcachon (A), Giglio, Mediterranean (G), Majorca, Mediterranean (M), Crete, Mediterranean (C), Hurghada, Red Sea (H), Florida (F), Phuket, Thailand (T). Only bootstrap values above 70 are indicated.

have yet been analyzed. Migration from the Red Sea would probably imply that in a very short time, about 100 years, the species extended its range through the entire Mediterranean Sea to the Atlantic. Immigrations of polychaetes can

occur extremely rapidly, as has been demonstrated for species of *Marenzelleria* that became distributed in the North and Baltic Seas within a decade (Bastrop *et al.*, 1997); however, these spionids have long-lived pelagic larvae



(Bochert, 1997), whereas *H. gohari* is thought to undergo direct development (Westheide, 1970). Along-shore migration is presumably a general feature of the latter species, but the rate of geographic expansion achieved by this means is unknown.

*H. gohari* is considered to be one of the many cosmopolitan interstitial polychaete species (Westheide, 1971; Sterrer, 1973; Westheide and Rao, 1977; Riser, 1981; Giere, 1993; Soosten *et al.*, 1998). As yet no morphological distinctions have been found among individuals from the most diverse of the earth's marine regions (see Fig. 1), so that the species must temporarily be regarded as distributed worldwide on the coasts of warm seas (Westheide, 1977; Hartmann-Schröder, 1991) provided that a morphological species concept is applied. In the present genetic study, all the cluster analyses employed show clear differences between the animals in the regions (1) European Atlantic/Mediterranean/Red Sea, (2) Indian Ocean, and (3) Western Atlantic (North America). Whether the observed genetic distances reflect species differences—that is, reproductive barriers—cannot be decided on the basis of current observations. In another cosmopolitan morphospecies, *Petitia amphophthalma* Siewing, 1956 (Syllidae), the distances found by RAPD analyses between North American and European animals correspond to the values found here: 0.60–0.66 (Soosten *et al.*, 1998). However, similar distance values (after Nei and Li, 1979) of geographically separated but morphologically differentiated pairs of interstitial polychaete species have been revealed by RAPD analyses as well: *Nerilla antemata* Schmidt, 1848–*N. mediterranea* Schlieper, 1925 (Nerillidae): 0.74–0.77 (Schmidt and Westheide, 1998); *Microphthalmus carolinensis* Westheide and Rieger, 1987–*M. nahantensis* Westheide and Rieger, 1987: 0.77 (unpubl. data). Schirmacher *et al.* (1998) found a distance value of only 0.17 between two enchytraeid sibling species. Thus the notion of *H. gohari* representing a cosmopolitan species will have to be abandoned.

It is remarkable in this connection that the Florida population, identified as *H. gohari*, in all analyses formed a sibling cluster with the freshwater species *H. riegerorum* Westheide, 1979. These animals are the nearest neighbors geographically to the latter species, with recorded finds on the East Coast of the United States in Florida (Westheide, 1995) and North Carolina; that is, they live only about 100 km away from the habitat of the freshwater species, which so far has been found only at a site in the sandy bank of the Chowan River in North Carolina. When *H. riegerorum* was first described, mention was made of its close morphological resemblance to *H. gohari* (Westheide, 1979). There is much evidence that the freshwater form separated from the neighboring marine *Hesionides* population on the seacoast. The genetic distance between *H. riegerorum* and the marine *Hesionides* individuals on the Florida coast is appreciable,

although considerably less than that between the latter and the *gohari* individuals from Thailand or Europe. But whereas the freshwater species is also clearly distinguishable morphologically (*e.g.*, by the different notopodial chaetae, penis papillae, and anal lobes; Westheide, 1979), no such diagnostic characters have yet been identified for the populations classified as *H. gohari*. The small geographic distance between the new freshwater species and its marine population of origin has evidently produced morphological character shifts, some of which may act as isolating mechanisms—for instance, the differently positioned and shaped penis papillae on the prostomium (see also Westheide, 1984, fig. 8). In contrast, the large geographic distance separating the European/Egyptian, Thai, and North American individuals from one another is correlated with genetic distinctions but not with any morphological ones that can readily be discerned, perhaps because of the stability of this marine habitat.

To continue to regard all these populations as belonging to a single species, *H. gohari*, is problematic. They would then constitute something like a paraphylum (Lorenzen, 1976), with one population (Florida) more closely related to the distinct sibling species (*H. riegerorum*) than to the other populations. As soon as possible, therefore, at least the North American "*H. gohari*" should be given the status of a separate species. However, we prefer not to undertake its description until the animals on the coast of the United States have been further examined in a specific search for morphological diagnostic characters, so that the practical taxonomic work will not be hampered by a species analysis based exclusively on genetic tests and on the geographic situation.

Regarding Sterrer's (1973) hypothesis, it is irrelevant whether these genetically derived clades represent distinct species or merely populations of a single, and hence indeed cosmopolitan, species. Testing this question, however, demands a larger number of specimens from different sites separated by oceans and sequencing techniques.

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### Literature Cited

- Armonies, W. 1989. Meiofaunal emergence from intertidal sediment measured in the field: significant contribution to nocturnal planktonic biomass in shallow waters. *Helgol. Meeresunters.* **43**: 29–43.
- Bastrop, R., M. Röhner, C. Sturmhuber, and K. Jürss. 1997. Where did *Marenzelleria* spp. (Polychaeta: Spionidae) in Europe come from? *Aquat. Ecol.* **31**: 119–136.
- Bochert, R. 1997. *Marenzelleria viridis* (Polychaeta: Spionidae): a review of its reproduction. *Aquat. Ecol.* **31**: 163–175.
- Büscher, N., E. Zyprian, and R. Blaiich. 1993. Identification of grapevine cultivars by DNA analyses: pitfalls of random amplified polymorphic DNA techniques using 10mer primers. *Vitis* **32**: 187–188.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 777–7783.
- Gerlach, S. A. 1977. Means of meiofauna dispersal. In *The Meiofauna Species in Time and Space*. W. Sterrer and P. Ax, eds. Workshop Symposium, Bermuda, 1975. *Mikrofauna Meeresboden* **61**: 89–103.
- Giere, O. 1993. *Meiobenthology. The Microscopic Fauna in Aquatic Sediments*. Springer, Berlin.
- Hadrys, H., M. Balick, and B. Schierwater. 1992. Application of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol.* **1**: 55–63.
- Hagerman, G. M., and R. M. Rieger. 1981. Dispersal of benthic meiofauna by wave and current action in Bogue Sound, North Carolina, USA. *Publ. St. Zool. Napoli (I. Mar. Ecol.)* **2**: 245–270.
- Hartmann-Schröder, G. 1960. Polychaeten aus dem Roten Meer. *Kieler Meeresforsch.* **16**: 69–125.
- Hartmann-Schröder, G. 1991. Die Polychaeten der subtropisch-tropischen bis tropischen Ostküste Australiens zwischen Maclean (New South Wales) und Gladstone (Queensland) sowie von Heron Island (Großes Barrier Riff). In: G. Hartmann-Schröder and G. Hartmann. Zur Kenntnis des Eulitorals der australischen Küsten unter besonderer Berücksichtigung der Polychaeten und Ostracoden. Teil 16. *Mitt. Hambg. Zool. Mus. Inst.* **88**: 17–71.
- Ile, Q., M. K. Viljanen, and J. Mertsola. 1994. Effects of thermocyclers and primers on the reproducibility of banding patterns in random amplified polymorphic DNA analysis. *Mol. Cell. Probes* **8**: 155–160.
- Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pääbo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* **86**: 6196–6200.
- Lorenzen, S. 1976. Zur Theorie der phylogenetischen Systematik. *Verh. Dtsch. Zool. Ges.* **1976**: 228.
- Nei, M., and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Genetics* **76**: 5269–5273.
- Palmer, M. A. 1988. Dispersal of marine meiofauna: a review and conceptual model explaining passive transport and active emergence with implications for recruitment. *Mar. Ecol. Prog. Ser.* **48**: 81–91.
- Peer, Y. van de, and R. de Wachter. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput. Appl. Biosci.* **10**: 569–570.
- Por, F. D. 1978. *Lessepsian Migration. The Influx of Red Sea Biota into the Mediterranean by Way of the Suez Canal*. Springer, Berlin 228 pp.
- Rao, G. C. 1972. On the geographical distribution of interstitial fauna of marine sand. *Proc. Indian Natl. Sci. Acad.* **38B**: 164–178.
- Remane, A., and E. Schulz. 1964. Strandzonen des Roten Meeres und ihre Tierwelt. *Kieler Meeresforsch.* **20**: 5–17.
- Riser, N. W. 1981. The aberrant polychaete *Stygocapitella* from some American beaches. *Wasmann J. Biol.* **38**: 10–17.
- Ruppert, E. E. 1977. Zoogeography and speciation in marine Gastrotricha. In *The Meiofauna Species in Time and Space*. W. Sterrer and P. Ax, eds. Workshop Symposium, Bermuda, 1975. *Mikrofauna Meeresboden* **61**: 231–251.
- Saitou, N., and M. Nei. 1987. The neighbour joining method. A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.
- Schierwater, B. 1995. Arbitrarily amplified DNA in systematics and phylogenetics. *Electrophoresis* **16**: 1643–1647.
- Schirmacher, A., H. Schmidt, and W. Westheide. 1998. RAPD-PCR investigations on sibling species of terrestrial *Enchytraeus* (Annelida: Oligochaeta). *Biochem. Syst. Ecol.* **26**: 27–36.
- Schmidt, H., and W. Westheide. 1998. RAPD-PCR experiments confirm the distinction between three morphologically similar species of *Nerilla* (Polychaeta: Nerillidae). *Zool. Anz.* **26**: 277–285.
- Sneath, P. H. A., and R. R. Sokal. 1973. *Numerical Taxonomy*. W. H. Freeman, San Francisco.
- Soosten, C. von, H. Schmidt, and W. Westheide. 1998. Genetic variability and relationships among geographically widely separated populations of *Petitia amphophthalma* (Polychaeta: Syllidae): Results from RAPD-PCR investigations. *Mar. Biol.* **131**: 659–669.
- Sterrer, W. 1973. Plate tectonics as a mechanism for dispersal and speciation in interstitial sand fauna. *Neth. J. Sea Res.* **7**: 200–222.
- Todaro, M. A., J. W. Fleeger, Y. P. Hu, A. W. Hrnicevich, and D. W. Foltz. 1996. Are meiofaunal species cosmopolitan? Morphological and molecular analysis of *Xenotrichula intermedia* (Gastrotricha: Chaetonotida). *Mar. Biol.* **125**: 735–742.
- Westheide, W. 1967. Monographie der Gattungen *Hesionides* Friedrich und *Microphthalmus* Mecznirow (Polychaeta). Ein Beitrag zu Organisation und Biologie psammobionter Polychaeten. *Z. Morph. Tiere* **61**: 1–159.
- Westheide, W. 1970. Zur Organisation, Biologie und Ökologie des interstitiellen Polychaeten *Hesionides gohari* Hartmann-Schröder (Hesionidae). *Mikrofauna Meeresboden* **3**: 1–37.
- Westheide, W. 1971. Interstitial Polychaeta (excluding Annelida). *Smithson. Contrib. Zool.* **76**: 57–70.
- Westheide, W. 1972/73. Nouvelles récoltes d'annélides interstitielles dans les plages sableuses du Bassin d'Arcachon. *Vie Milieu Serie A* **23**: 365–370.
- Westheide, W. 1972a. Räumliche und zeitliche Differenzierungen im Verteilungsmuster der marinen Interstitialfauna. *Verh. Dtsch. Zool. Ges.* **1971**: 23–32.
- Westheide, W. 1972b. La faune des Polychètes et des Archiannélides dans les plages sableuse à ressac de la côte méditerranéenne de la Tunisie. *Bull. Inst. Natl. Sci. Tech. Oceanogr. Peche, Salammbô* **2**: 449–468.
- Westheide, W. 1977. The geographic distribution of interstitial polychaetes. In *The Meiofauna Species in Time and Space*. W. Sterrer and P. Ax, eds. Workshop Symposium, Bermuda, 1975. *Mikrofauna Meeresboden* **61**: 287–302.
- Westheide, W. 1979. *Hesionides riegerorum* n. sp., a new interstitial freshwater polychaete from the United States. *Int. Rev. Gesamten Hydrobiol.* **64**: 273–280.
- Westheide, W. 1984. The concept of reproduction in polychaetes with small body size: adaptations in interstitial species. In *Polychaete Reproduction*. A. Fischer and H.-D. Pfannenstiel, eds. *Fortsch. Zool.* **29**: 265–287.
- Westheide, W. 1990. Polychaetes: Interstitial Families. In *Synopsis of the British Fauna (New series)* No. 44, D. M. Kermack and R. S. K. Barnes, eds. Universal Book Services/W. Backhuys, Oegstgeest, The Netherlands.
- Westheide, W. 1991. The meiofauna of the Galapagos. A review. Pp.

- 37–73 in *Galapagos Marine invertebrates. Taxonomy, Biogeography, and Evolution in Darwin's Islands*, M. J. James, ed. Plenum, New York.
- Westheide, W. 1992.** Neue interstitielle Polychaeten (Hesionidae, Dorvilleidae) aus dem Litoral des Golfs von Bengalen. *Microfauna Mar.* **7**: 147–157.
- Westheide, W. 1995.** *Pisione hartmannschroederiae* sp.n. (Polychaeta: Pisionidae) from a Florida sand beach. *Mitt. Hambg. Zool. Inst.* **92**: 77–84.
- Westheide, W., and G. G. Rao. 1977.** On some species of the genus *Hesionides* (Polychaeta, Hesionidae) from Indian sandy beaches. *Cah. Biol. Mar.* **18**: 275–287.
- Westheide, W., and R. M. Rieger. 1987.** Systematics of the amphiatlantic *Microphthalmus-listensis*-species-group (Polychaeta: Hesionidae): facts and concepts for reconstruction of phylogeny and speciation. *Z. Zool. Syst. Evolutionsforsch.* **25**: 12–39.
- Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990.** DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* **18**: 6531–6535.